Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA)
Available online at www.inia.es/forestsystems
http://dx.doi.org/10.5424/fs/2013221-02084

Forest Systems 2013 22(1), 3-14
ISSN: 2171-5068
eISSN: 2171-9845

The effect of water availability on plastic responses and biomass allocation in early growth traits of *Pinus radiata* D. Don

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Abstract

Aim of study: The aim of the study was to assess the effect of water availability on plastic responses and biomass allocation in early growth traits of *Pinus radiata* D. Don.

Area of study: Seedlings of 69 families of *P. radiata* belonging to five different sites in Central Chile, ranging from coastal range to fothills of the Andes, were grown in controlled conditions to evaluate differences in response to watering.

Material and methods: The seedlings were subjected to two watering regimes: well-watered treatment, in which seedlings were watered daily, and water stress treatment in which seedlings were subjected to three cyclic water deficits by watering to container capacity on 12 days cycles each. After twenty-eight weeks root collar diameter, height, shoot dry weight (stem + needles), root dry weight, total dry weight, height/diameter ratio and root/shoot ratio were recorded. Patterns and amounts of phenotypic changes, including changes in biomass allocation, were analyzed.

Main results: Families from coastal sites presented high divergence for phenotypic changes, allocating more biomass to shoots, and those families from interior sites presented low phenotypic plasticity, allocating more biomass to roots at the expense of shoots. These changes are interpreted as a plastic response and leads to the conclusion that the local landrace of *P. radiata* in Chile originating from contrasting environments possess distinct morphological responses to water deficit which in turn leads to phenotypic plasticity.

Research highlights: Families belonging to sandy soil sites must be considered for tree breeding in dry areas, selecting those with high root:shoot ratio.

Key words: early testing; environmental interaction; ontogeny; plasticity index; water stress.

Introduction

Plant species introduced outside their native ranges may face very different environmental conditions than those in their native habitats. Soil properties, hydrology, or growing season can all differ. Successful establishment and subsequent spread depends on its ability to tolerate and adapt to the new environmental scenarios and selective pressures. One way plants will respond to these changes is through environmentally induced shifts in phenotype, *i.e.* phenotypic plasticity (the ability of a genotype to alter its morphology and physiology in response to changes in the environmental conditions) (Bradshaw, 1965; Nicotra *et al.,* 2010), which allows species to grow in a range of environments (Corcuera *et al.,* 2010). Differences in the environmental heterogeneity have been postulated to conduct to divergences in plasticity between the genotypes adapted to stable *versus* changing habitats (*e.g.* Balaguer *et al*., 2001). It is well known that native plants from sites with contrasting soil and climatic differences develop phenotypic plasticity (Calamassi *et al*., 2001; Zhang *et*

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Received: 23-08-11. Accepted: 24-10-12.

Abbreviations used: choice experiment (CE or CEs); conditional logi (CL); contingent valuation (CV or CVs); independently and identically distributed (IID); independence of irrelevant alternatives (IIA); multinomial probit model (MNP); random parameters logit model (RPL); status quo (SQ).

al., 2004; Lei *et al*., 2006; Merchant *et al*., 2007). This has also been observed in introduced species with a limited number of generations in their new environment (Danusevičius and Persson, 1998), which is possible because phenotypic plasticity can potentiate rapid evolutionary change (Behera and Nanjundiah, 2004).

Radiata pine (*Pinus radiata* D. Don) plantations comprise 75 percent of the Chilean forest industry base, earning foreign currency on the order of US$ 5 billion in exports (INFOR, 2010). The rapid growth of the plantations (15 to 36 m^3 ha^{-1} $year^{-1}$) (Toro and Gessel, 1999) has been a key factor in the mass plantation of this species. Radiata pine was introduced in Chile during the 1890s. It was initially established in the city of Concepción (Camus, 2006), and after faster tree growth compared to the growth in its native California was recognized, a process of expansion into other areas of the country was commenced. Nowadays, with five generations of growth and 1.46 million ha of estate, is the most extensively planted conifer in the country. Studies of the ecological factors that influence the productivity of radiata pine have been carried out (Gerding and Schlatter, 1995; Huber and Trecaman, 2002). In each of these studies, climatic and soil characteristics were considered to be the most important factors determining the productivity of the species. It is largely known that drought constraints on forest yields will increase in importance as climate change leads to increasingly hotter and drier summers, which in turn will affect survival and productivity of forest trees. In Chile, despite an increase in drought is expected in the future (a decline of up to 30% in the productivity of *P. radiata* plantations is expected CONAMA 2009; Bahamóndez *et al*., 2010), genetic improvement for *P. radiata* has been directed towards characters such as volume, form and wood density. There is a lack of intensive studies on plastic responses for early growth traits to drought, as a tool to explore the potential of early testing in the Chilean radiata pine breeding programme. An incomplete understanding of the mechanisms that make certain genotypes grow faster even when faced with drought conditions has prevented defining selection criteria for tree improvement. Therefore, investigations into early plastic responses and their interaction with water availability are required.

Plasticity has been observed for different native and introduced forest species subjected to different abiotic stresses (Kaufman and Smouse, 2001; Atzmon *et al*., 2004; Chambel *et al*., 2007; Merchant *et al*., 2007; Aranda *et al*., 2010). In Australia, for radiata pine seedlings and adult trees, different responses to biotic stresses have been observed (Dean and Sands, 1983; Rowell *et al*., 2009), while for native populations of the species, variation in resistance to pathogens, pests, animals and cold were also observed (Hood and Libby, 1980; Simpson and Ades, 1990).

Given the history of its rapid expansion in Chile following its introduction, new radiata pine plantations have certainly experienced different selection pressures, resulting in adaptation to new conditions. The species were initially established in coastal sites, with site conditions similar to those found in its native range, and after a couple of generations, new plantations were established in sandy soils sites, with water holding capacity as the most important factor limiting growth (Albert, 1900; Lewis and Ferguson, 1993; Huber and Trecaman, 2002; Camus, 2006). The goal of this paper is to test whether populations of radiata pine from coastal sites show different responses to drought than populations from sandy soil sites. According to the optimum allocation hypothesis, these different responses would result in (1) differential growth in terms of biomass accumulation and (2) higher investment in below-ground biomass. It is expected that families from sandy soils allocate more biomass to roots at the expense of shoots. To address these questions, phenotypic, growth, and biomass partitioning changes, in response to two watering regimes, were analyzed in young seedlings from 56 families belonging to five growth sites of radiata pine covering most of the geographic distribution of the species in Chile. The specific objectives were: (1) to assess the variability in morphology between well-watered and water stress *P. radiata* seedlings, (2) to determine the pattern of biomass allocation and (3) to examine the degree of plastic response to water regimes.

Methods

Material

We used a subset of the first generation open-pollinated families of the radiata pine breeding programme in Chile. Seeds from these families were provided by Forestal Celco S.A. and Forestal Mininco S.A., and were randomly selected from seed orchards of these companies. Seed originated from 25 year old plus trees selected between 1976-1978 for traits such as superior

Table 1. Origin of radiata pine investigated: soil and climatic features of their zones of origin

Plus-trees origin (site)	Anual rainfaill (mm)	Summer relative humidity (%)[1]	Sumer maximum temperature (°C)[1]	Winter rainfall (mm)[2]	Parent material	Water holding capacity (mm)	Location		Number of families
							Latitude S	Longitude W	
Eight Region Sandy Soils	1,100-1,208	63-65	26.1-28.6	553-619	Sandy	100	37° 31’	72° 04’	21
Seventh Region Coastal	450-950	70-80	18.6-24.7	460-508	Granitic-metamorphic	400	35° 21’	72° 27’	18
Eight Region Coastal	1,300	77-80	19.4-23.9	588-713	Granitic-metamorphic	400	38° 14’	73° 17’	27
Eight Region Fothills of the Andes	1,600	60-65	19.6 - 25.9	900	Volcanic ash	400	36° 39’	71° 40’	5
Eight Region Central Valley	1,000-1,300	60-65	26.0-27.2	433-500	Red clay	400	36° 56’	72° 22’	9

[1] Summer relative humidity and maximum temperature include January and February. [2] Winter rainfall include June, July and August.

growth, stem form and volume, at five different sites in Central Chile, ranging from Coastal Range, across Central Valley to Fothills of the Andes in the Seventh and Eight Regions (Table 1, Fig. 1).

Nursery experiment

Seeds from 80 open-pollinated families were sown on 140 cm^3 containers filled with a mixture of composted bark of radiata pine and perlite (8:2 v), combined with the slow-release fertilizer Basacote™ plus 6M. Seeds were submerged in distilled water for 24 hour before sowing. The experimental material was raised in the Catholic University nursery at Talca city (35° 26’ S, 71° 37’ 13” W, 131 m of elevation) for twenty weeks under normal watering regimes (watering every day). Average temperature and relative humidity fluctuated between 14-20°C and 60-70% respectively. From the 80 families sowed, and probably due to the age of the seeds, only 69 families germinated enough seedlings to perform the experiment and run statistical analysis. Seven weeks after sowing, those 69 families were placed following a split-plot design, with watering regime as the whole plot and family nested within site as the sub-plots. Five sites of origin, 69 families and two watering treatments were used. The two watering treatments, well watered (WW) and water stress (WS), were applied to three replicates of 69 families, with 16

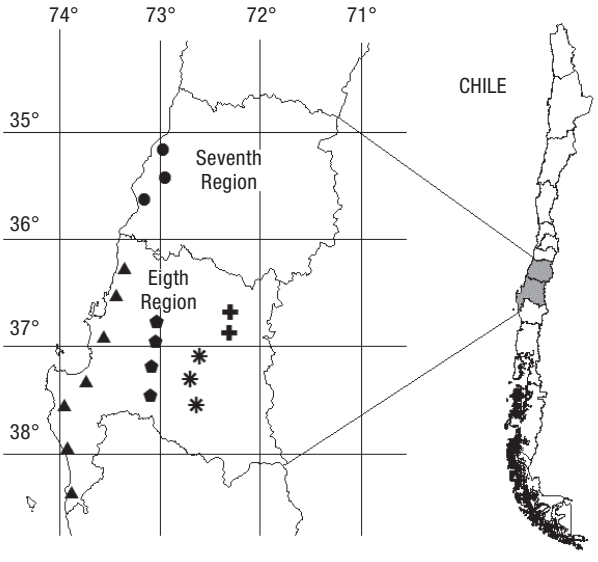

Figure 1. Location of the *P. radiata* plus-tress collected between 1976-1978 (∗ = Families from Sandy Soils Eight Region, ● = Families from Seventh Region Coastal Sites, ▲ = Families from Eight Region Coastal Sites, ✚ = Families from Eight Region Fothills of the Andes Sites, ⬟ = Families from Eight Region Central Valley Sites).

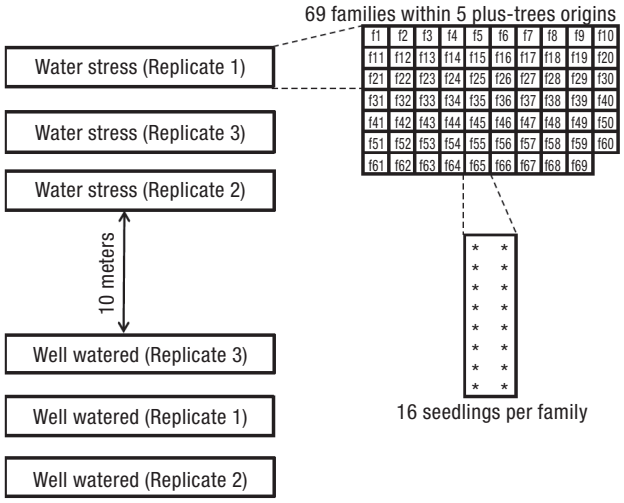

Figure 2. Experimental split-plot layout for 69 successfully germinated families. Family plots were randomized within replications.

seedlings of each family included per sub plot (Fig. 2). The watering treatments were not applied to individual families; instead they were applied to trays containing 6 families each. The efficiency of an incomplete block design, relative to design with full replicates was calculated under the same conditions, but no additional efficiency was achieved by blocking. Local microenvironmental conditions were strictly controlled (*i.e.* wind, position of the trays with respect to the solar angle, and drift of water mist), in order to allow all seedlings to receive the same growth conditions, except for the watering regime.

Twenty weeks after sowing, seedlings from the 69 families were subjected to two watering regimes based on predawn plant water potentials (Ψ_{pd}): (1) well-watered treatment, in which seedlings were watered daily to container capacity to maintain a Ψ_{pd} of -0.5 ± 0.1 MPa, as measured with a pressure chamber. (2) cyclic water stress treatment in which seedlings were subjected to cyclic water deficits by watering to container capacity on three 12-days cycles when Ψ_{pd} reached -1.5 ± 0.1 MPa on average. Water potential was measured in the three 12-days cycles by using a subsample of three seedlings per six families per watering regime, and was only used to validate a reference value, obtained from bibliography, to apply the withholding of water. According to Squire *et al*. (1987), with ten days of withholding of water, five-month old *P. radiata* seedlings reach a water potential of –1,9 MPa with seedlings been wilted. Temperature and relative humidity averaged 22°C and 59% respectively. During this period, the water supply was withdrawn until the water content of each tray (with 88 containers and 6 families each) reached 40% of field capacity (determined by weight). Water availability was homogeneous among families, and this watering level was maintained approximately constant until the end of the stress period. After the water restriction was applied, only 56 families with at least 15 seedlings survived in the water stress treatment. The material used to perform the analysis consisted of 15 seedlings per family and watering regime.

Assessments

The seedlings were harvested eight weeks after water-stress treatment started. Total height (H), root collar diameter (D), shoot dry weight (stem + needles) (SDW) and root dry weight (RDW) were measured (80°C, 24 h). The total dry weight (TDW = SDW + RDW), the root/shoot ratio (RSR = RDW/SDW) and the height/diameter ratio (HD = H/D) were also calculated. Fifteen seedlings per water regime treatment and per family were harvested. All weight and linear measurements are in milligrams (mg) and millimeters (mm), respectively.

Statistical analysis

Growth (H, D, HDR) and biomass (TDW, RDW, SDW, RSR) traits were analyzed with the general linear model approach (GLM) to analysis of variance, with type III sum of squares, using SAS software (SAS Institute, 1999). Prior to the analysis, the data were examined and conformed to the normality and homogeneity of variance assumptions required for the analysis of variance. The model terms were fitted according to the hierarchical design of the experiment, considering families as nested within sites [1].

$$Y_{ijkl} = \mu + W_i + O_j + F(O)_{kj} + W_i * F(O)_{kj} + e_{ijkl} \quad [1]$$

where Y_{ijkl} is the observed phenotypic measurement (growth and biomass traits), μ is the overall mean, W_i is the fixed effect of i^{th} watering regime, O_j is the fixed effect of j^{th} plus-tree origin, $F(O)_{kj}$ is the random effect of the k^{th} family nested within the j^{th} plus-tree origin, $W_i*F(O)_{kj}$ is the interaction between i^{th} watering regime and the k^{th} family nested within the j^{th} plus-trees origin, and e_{ijkl} is the experimental random error. A significant effect of the water stress treatment in this analysis indi-

cates the existence of phenotypic changes in response to drought for the trait considered, and a significant genotype X environment interaction [i.*e.* interaction W_i*$F(O)_{kj}$ of the model], indicates the existence of differences among families within sites for those changes. Whenever the treatment factor was significant, the difference between mean phenotype of each family within sites in the two watering regimes considered was evaluated with a t-test.

For the plasticity analysis the position of each population in the space defined by its mean phenotype under the water stress treatment (on the x-axis) and under the well-watered treatment (on the y-axis) was represented graphically following Pigliucci and Schlichting (1996). This way, each family is represented by a single point and, if the two axes are in the same scale, the main diagonal represents the line of null phenotypic change, that corresponds to a flat reaction norm and the tangent (slope) of the angle α, formed between the line connecting each point to the origin and the x-axis can be interpreted as an index quantifying phenotypic change. The main advantage of this index, when compared to the most common methods based on the difference between mean phenotypic values in each environment (*e.g.* Scheiner, 1993), represented in this biplot by the orthogonal distance to the main diagonal, is that the slope is reflecting the change in relative terms, more significant from a biological point of view. Besides, this index also reflects the direction of the response (slope higher or lower than one), which has obvious biological relevance (Via, 1993). We will further refer to this index as angular phenotypic change index (APCI).

For the biomass allocation analysis, we performed an allometric analysis through the regression of the natural logarithms of each biomass component (RDW and SDW) (Poorter and Nagel, 2000). The classical allometric equation was used:

$$Y = \alpha X^{\beta} \qquad [2]$$

where β is the allometric exponent and α is the allometric coefficient. The allometric equation was log-transformed to yield a simple linear relationship:

$$Y = \alpha X^{\beta} \leftrightarrow \log(Y) = \log(\alpha) + \beta \log(X) \qquad [3]$$

where the allometric exponent becomes the slope and the log of the allometric coefficient is the intercept. Changes in allocational patterns were assessed by comparison of the slopes and intercepts corresponding to different watering levels (Schlichting and Pigliucci, 1998). When, for a given family within site, a strong linear relation between biomass compartments existed and the two lines of regression corresponding to the two water treatments overlapped, the slope of those lines will differ only if the water stress treatment caused significant changes in the relative growth rates of shoots and roots. Following Samson and Werk (1986) and Klinkhamer *et al*. (1990), F-tests were used to investigate proportionality of allocation and the influence of water on these relationships. This was done using RDW as the dependent variable (Y) and SDW as the covariate (X) in the GLM.

Results

Growth and biomass response to water stress

Despite germination was variable in all 80 families initially sowed, no statistical differences were detected ($p < 0.01$) for germination. Only two families, from Seventh and Eight Region Coastal Sites, germinated less than 15 seedlings form 96 seedlings sowed (*i.e.* less than 20% germination). However 11 families were discarded due to low number of seedlings to perform statistical analysis, giving a total of 69 families to perform the watering regime treatments. This could be possibly due to different storage conditions of this families (*i.e.* differences in moisture content), which could have affected respiration and assimilation, and consequently loss of vigor. Seeds have been in storage since 1978, and unfortunately there is no information about storage conditions (*i.e.* moisture content and temperature). On the other hand, initial plant size did not influence any of the plant growth or biomass traits. Before starting the water stress treatment root collar diameter and height were homogeneous for all families under study ($p < 0.01$). At 20 weeks of growth an average of 10.4 ± 0.12 cm in height and 1.85 ± 0.02 mm in diameter was observed. This indicates that the observed response in all families after water stress is more attributable to the effect of the withholding of water than the ontogenetic state of the seedlings.

The level of water supplied to the plants affected the growth of the families of radiata pine in all variables. The low water potential at dawn (average -1.5 ± 0.1 MPa) in the water stress treatment was sufficient to generate a negative growth response in plants under study, exhibiting a strong growth reduction (Table 2). All traits were significantly reduced during the drought period in the stressed plants of all 56 surviving families ($p < 0.01$).

Table 2. Different morphological traits among 56 half-sibs families of radiata pine growing in different watering regimes (mean ± S.E. of the mean; n = 1,680)

Trait	Watering regime	
	Well-watered	Water stress
H (mm)	137.26 ± 0.09[a]	119.28 ± 0.09[b]
D (mm)	2.58 ± 0.01[a]	1.98 ± 0.01[b]
HDR	5.37 ± 0.03[a]	6.11 ± 0.04[b]
RSR	1.14 ± 0.00[a]	1.11 ± 0.00[b]
SDW (mg)	2,060.11 ± 0.01[a]	1,768.41 ± 0.00[b]
RDW (mg)	1,805.11 ± 0.01[a]	1,600.43 ± 0.00[b]
TDW (mg)	3,865.12 ± 0.02[a]	3,368.81 ± 0.01[b]

H: total height. D: root collar diameter. HDR: height/diameter ratio. RSR; root/shoot ratio. SDW: shoot dry weight. RDW: roots dry weight. TDW; total dry weight. Different letters indicate statistical differences for watering regime at $p < 0.01$.

Differences due to site of origin of the families or the family nested within the site of origin for most variables were not observed when analyzed independently. For the two watering regimes, differences for all variables were also observed. When families within sites interacting with watering regime were analyzed *i.e.* interaction W*F(O) in equation [1]), significant differences for all traits were observed (Table 3). Families from some sites (*e.g.* Eight Region Sandy Soils and Eight Region Fothills of the Andes sites) developed more diameter and height in the water-stress treatment. The same situation was observed in the case of RDW, SDW and TDW. In the well-watered treatment, families belonging to Seventh and Eight Region Coastal sites had a better performance in growth traits (Table 4). Watering regime accounted for the highest proportion of the variability encountered in the analysis of most traits, as expected, reaching up to 23% for D (Table 3). Nevertheless, despite watering by family within site interaction explained only a minor percentage of the variance (*i.e.* up to 9.7% for RSR), its effect was highly significant for all the variables analyzed. Family nested within site was not significant either for D and or dry masses, but it was significant for H ($p < 0.01$) and the height-related trait HDR (Table 3). These three factors account for up to 36% of the variability.

Inter-site plasticity variation

After ANOVA was performed, APCI values analysis reflected differences in plasticity for almost traits analyzed. For growth traits (D, H), families belonging to coastal sites showed the highest values for APCI, ranging from 0.97 for D and 0.86 for H. By contrast, families from interior sites (*i.e.* Eight Region Fothills of the Andes and Eight Region Sandy Soils sites) were relatively stable, since its angular plasticity index is lower and a little closer to the zero plasticity line. In the case of H, families from the Eight Region Fothills of the Andes sites exhibited the lowest plasticity values (Fig. 3, Table 5). Differences in plasticity were also observed within sites (*i.e.* between families), being families from the interior sites the less plastic, and families from coastal sites the more one.

For some of biomass traits (SDW, RSR), families belonging to coastal sites also showed the highest values for APCI, ranging from 0.81 for RSR, to 0.91 for SDW. On the other hand, families of Eight Region Sandy Soils and Eight Region Fothills of the Andes sites were relatively stable, since its plasticity index is lower and a little closer to the zero plasticity line. For all traits differences between sites were observed, in the case of SDW, families from the Fothills of the Andes Eight Region sites exhibited the lowest plasticity values (Fig. 4, Table5).

Table 3. Percentage of the variance due to watering regime (W), site of origin (O), family nested within site [F(O)], watering by site interaction (W*O) and watering by family nested within site interaction (W*F(O)), for growth and biomass traits

Source of variation[1]	df[2]	D		H		HDR		SDW		RDW		TDW		RSR	
		PV[3]	Sig.[4]	PV	Sig.	PV	Sig.	PV	Sig.	PV	Sig.	PV	Sig.	PV	Sig.
W	1	23.1	***	5.53	***	6.33	***	12.33	***	9.63	***	12.86	***	0.51	***
O	4	0.10	n.s	0.10	n.s	0.28	n.s	0.03	n.s	1.46	*	0.44	n.s	2.13	n.s
F(O)	51	7.38	n.s	10.78	**	11.37	***	7.74	n.s	8.36	n.s	7.30	n.s	11.69	n.s
W*O	4	1.22	*	0.42	n.s	0.74	*	0.09	n.s	0.18	n.s	0.04	n.s	0.69	n.s
W*F(O)	51	5.72	***	5.04	***	3.85	***	5.62	***	5.61	***	4.98	***	9.71	***

H: total height. D: root collar diameter. HDR: height/diameter ratio. RSR: root/shoot ratio. SDW: shoot dry weight. RDW: roots dry weight. TDW: total dry weight. [1] According to equation [1]. [2] Degrees of freedom. [3] Percentage of the variance (%). [4] Ssignificance levels (n.s: non significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

Table 4. Growth and biomass partitioning of *P. radiata* according to the plus-trees origin. Means ± standard errors (different letters indicate statistical differences for watering regime at $p < 0.01$)

Site	Watering regime	D (mm)	H (mm)	HDR	RDW (mg)	SDW (mg)	TDW (mg)	RSR
Eight region sandy soils	Well-watered	2.51 ± 0.02[a]	134.8 ± 1.81[a]	5.40 ± 0.06[a]	1,842.7 ± 20.6[a]	2,050.8 ± 23.1[a]	3,893.6 ± 39.8[a]	*1.12 ± 0.00[a]
	Water-stress	2.02 ± 0.02[b]	119.2 ± 1.67[b]	5.97 ± 0.07[b]	1,633.8 ± 16.7[b]	1,777.9 ± 17.3[b]	3,411.0 ± 31.9[b]	*1.09 ± 0.00[b]
Seventh region coastal	Well-watered	2.58 ± 0.03[a]	138.4 ± 2.05[a]	5.37 ± 0.05[a]	1,748.7 ± 19.6[a]	2,055.2 ± 27.5[a]	3,804.1 ± 44.3[a]	*1.17 ± 0.01[a]
	Water-stress	1.99 ± 0.02[b]	117.7 ± 2.01[b]	6.00 ± 0.09[b]	1,546.9 ± 13.8[b]	1,714.6 ± 18.6[b]	3,308.4 ± 28.8[b]	*1.14 ± 0.01[b]
Eight region coastal	Well-watered	2.65 ± 0.02[a]	138.8 ± 1.54[a]	5.29 ± 0.05[a]	1,795.1 ± 17.2[a]	2,068.1 ± 20.1[a]	3,863.2 ± 34.2[a]	1.16 ± 0.00[a]
	Water-stress	1.92 ± 0.02[b]	119.2 ± 1.55[b]	6.29 ± 0.08[b]	1,614.1 ± 15.2[b]	1,758.1 ± 13.7[b]	3,372.3 ± 26.0[b]	1.09 ± 0.00[b]
Eight region fothills of the Andes	Well-watered	2.42 ± 0.08[a]	130.9 ± 4.74[a]	5.45 ± 0.15[a]	1,887.0 ± 55.5[a]	2,039.6 ± 63.4[a]	3,926.7 ± 113.1[a]	1.08 ± 0.01[a]
	Water-stress	1.99 ± 0.06[b]	128.4 ± 5.46[a]	6.41 ± 0.14[b]	1,635.0 ± 38.7[b]	1,817.3 ± 48.1[b]	3,452.3 ± 79.1[b]	1.11 ± 0.02[a]
Eight region central valley	Well-watered	2.55 ± 0.04[a]	138.5 ± 3.06[a]	5.52 ± 0.13[a]	1,830.2 ± 31.9[a]	2,076.7 ± 36.3[a]	3,907.0 ± 64.4[a]	1.14 ± 0.01[a]
	Water-stress	2.01 ± 0.04[b]	119.8 ± 2.62[b]	6.05 ± 0.14[b]	1,574.1 ± 19.1[b]	1,774.0 ± 21.1[b]	3,348.1 ± 37.2[b]	1.13 ± 0.01[a]

H: total height. D: root collar diameter. HDR: height/diameter ratio. RSR: root/shoot ratio. SDW: shoot dry weight. RDW: roots dry weight. TDW: total dry weight. * $p < 0.05$.

Patterns of biomass allocation

In general, the fraction of biomass in aboveground organs (stems + needles) generally decreased, and fraction in roots increased with lower water availability. On the log-log-scale, significantly ($p < 0.01$) different slopes and intercepts in allometric regression lines for well-watered *vs.* water-stress treatments were found in three out of five sites for biomass allometry, these were Seventh and Eight Region Coastal and Eight Region Sandy Soils sites (Fig. 5). Besides, the interaction between the watering regime with family and with log of SDW as a covariate were significantly for the same three sites, which indicates that trajectories of biomass allocation were altered by watering regime and the covariate, *i.e.* RDW was not only affected by watering regime but also by SDW. In families from Seventh Region Coastal sites, more biomass was allocated to shoots in water-stress treatment, while in families from Eight Region Sandy Soils and Eight Region Coastal sites, more biomass was allocated to roots (Fig. 5).

Discussion

The present paper is focused on the morphological response of families from Chilean landrace of radiata pine to two contrasting watering regimes. Until present, little information was available on the morphological changes induced by water stress during the initial developmental stages of this species. It is largely known that in the case of low nutrient or water availability, there will be a decreased water uptake per unit root mass which is expected to increase the allocation

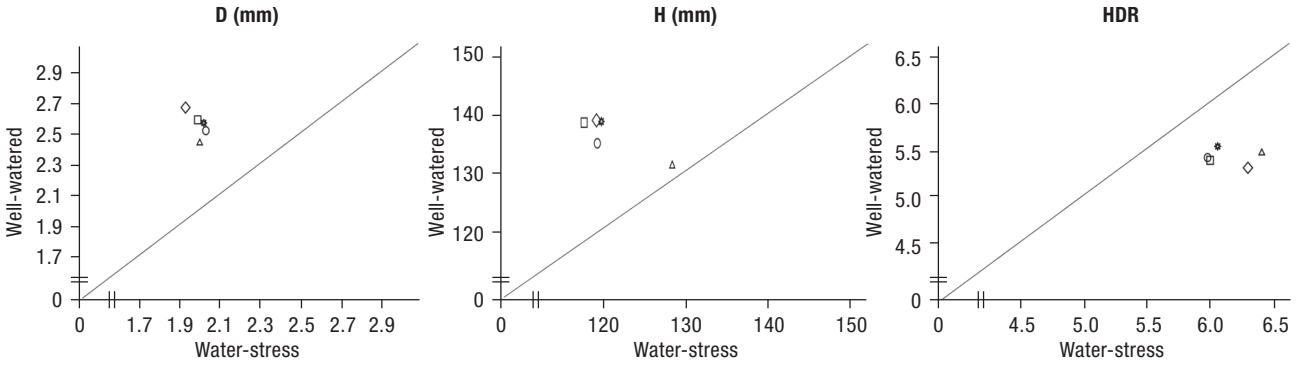

Figure 3. Angular Phenotypic Change Index for growth traits root collar diameter (D), height (H) and height/diameter ratio (HDR). (○ = Eight Region Sandy Soils Sites, □ = Seventh Region Coastal Sites, ◇ = Eight Region Coastal Sites, △ = Eight Region Fothills of the Andes Sites, ✻ = Eight Region Central Valley Sites).

Table 5. Angular Phenotypic Change Index of *P. radiata* families, according to the plus-trees origin (site). Means ± standard errors (different letters indicate statistical differences for site at $p < 0.01$)

Site	D	H	HDR	RDW	SDW	TDW	RSR
Eight region sandy soils	0.891 ± 0.013[a]	0.815 ± 0.013[a]	0.736 ± 0.011[a]	0.846 ± 0.011[a]	0.855 ± 0.012[a]	0.925 ± 0.009[a]	0.736 ± 0.012[b]
Seventh region coastal	0.923 ± 0,015[a]	0.876 ± 0,015[a]	0.739 ± 0.010[a]	0.843 ± 0.010[a]	0.890 ± 0,010[a]	0.936 ± 0.007[a]	0.799 ± 0.009[a]
Eight region coastal	0.973 ± 0.010[b]	0.864 ± 0.009[a]	0.800 ± 0.009[b]	0.838 ± 0.006[a]	0.916 ± 0.007[a]	0.935 ± 0.008[a]	0.812 ± 0.006[a]
Eight region fothills of the Andes	0.869 ± 0.034[a]	0.703 ± 0.034[b]	0.804 ± 0.002[b]	0.961 ± 0.005[b]	0.772 ± 0.034[b]	0.921 ± 0.020[a]	0.791 ± 0.030[a]
Eight region central valley	0.904 ± 0.021[a]	0.862 ± 0.025[a]	0.737 ± 0.009[a]	0.965 ± 0.015[b]	0.903 ± 0.020[a]	0.991 ± 0.017[b]	0.729 ± 0.009[b]

H: total height. D: root collar diameter. HDR: height/diameter ratio. RSR: root/shoot ratio. SDW: shoot dry weight. RDW: roots dry weight. TDW: total dry weight.

to roots. However, in the case of limited water supply, the changes are only modest (Poorter and Nagel, 2000). Results of this study are in agreement with this statement. Among the five sites of growth of radiata pine in Chile, the allometric trajectories for shoots and roots exhibited significant differences between water regimes, observing that only families from Eighth Region Sandy Soils and Eighth Region Coastal sites shifted allocation to roots when exposed to water tress.

In the analysis of variance, as expected, watering accounted for the highest proportion of the variability encountered in the analysis of most traits, which confirms the severity of the applied stress. Although families within sites explained only a minor percentage of the variance (from 7% to 11%), which still suggests a high family variability. At the same time, the interaction W*F(O) explained up to 9% of the variation, which indicates the existence of differences among families within sites (Table 3). These analyses confirmed differences in plasticity for all traits analyzed. For example, families belonging to the Sandy Soils of the Eighth Region (see Table 1), in which soils have a low water holding capacity and high vapor pressure deficit (Gerding and Schlatter, 1995; Huber and Trecaman, 2002), exhibited superior growth to the families from coastal areas when they were growing under water-

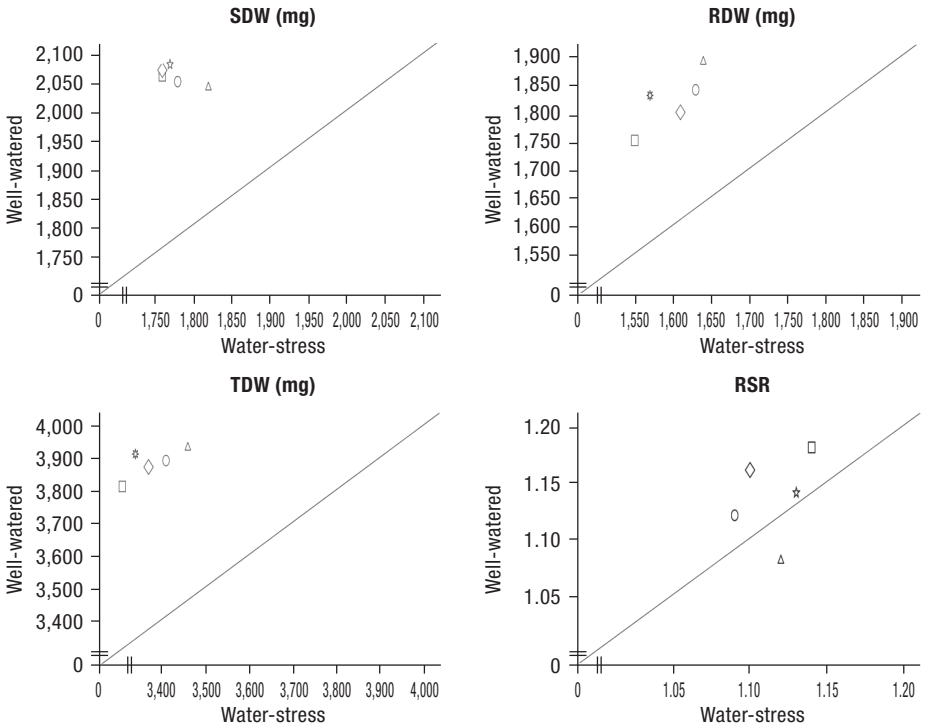

Figure 4. Angular Phenotypic Change Index for biomass partitioning traits shoot dry weight (SDW), root dry weight (RDW), total dry weight (TDW) and shoot/root ratio (RSR). (○ = Eight Region Sandy Soils Sites, □ = Seventh Region Coastal Sites, ◇ = Eight Region Coastal Sites, △ = Eight Region Fothills of the Andes Sites, ✻ = Eight Region Central Valley Sites).

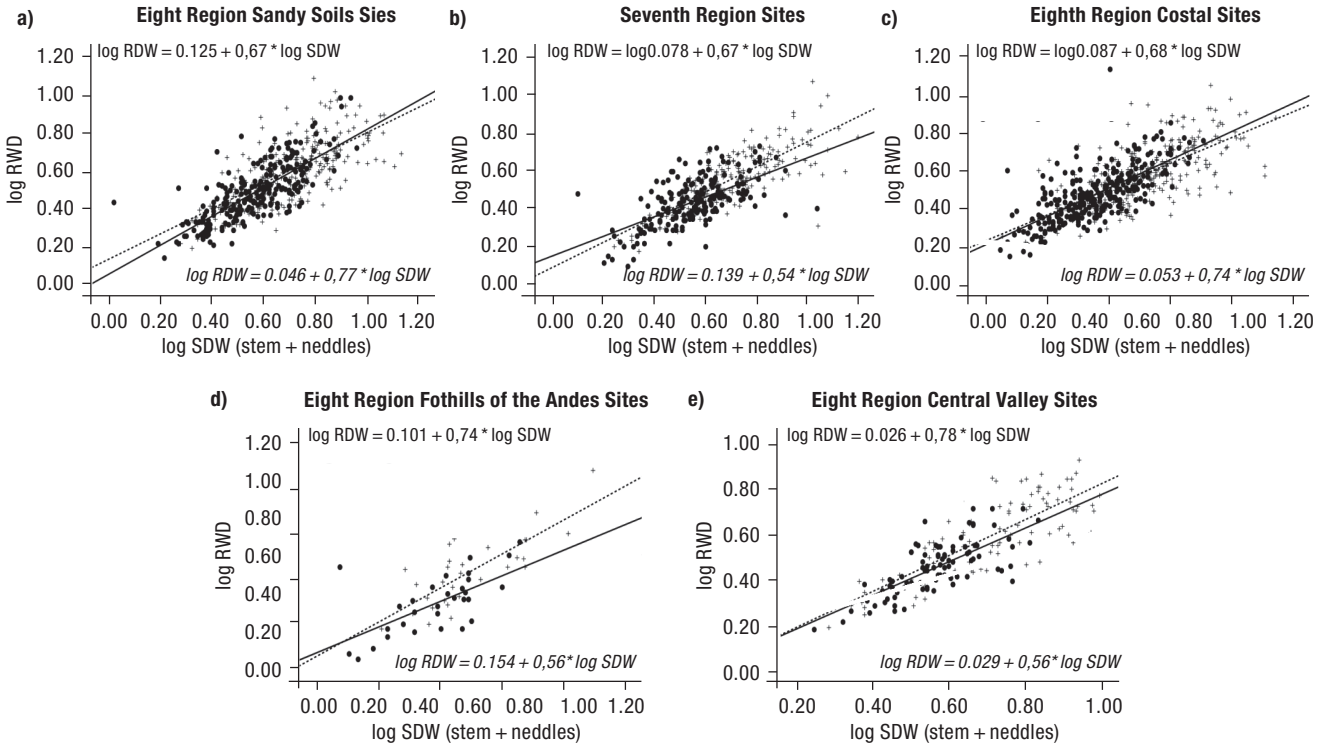

Figure 5. Effects of water treatment on the allometric relationship between root and shoot biomass of the five sites of origin of *P. radiata* studied. Black circles and continuous lines represent stressed plants, black crosses and dotted lines represent non-stressed plants. a,b,c) Significant differences ($p < 0.01$) in slopes/intercepts between watering regimes. d,e) No significant differences in slopes between treatments (Allometric regression lines in bold represents well-watered treatment, while in *italics* represents water-stress treatment).

stress and also allocated more biomass to roots in comparison to the other four sites (Table 4). Although they have a slightly less plasticity for shoot biomass (SDW), this situation is reversed slightly in the case of root biomass (RDW). Some organisms, particularly in the early stages of development, have the ability to alter their patterns of carbon allocation in response to certain environmental stimuli (Bloom *et al*., 1985). According to the theory of specialization (Lortie and Aarssen, 1996), compared with genotypes adapted to drought conditions, genotypes adapted to favorable environments present a superior performance in these environments, but this is reduced when conditions are limiting, which results in a high phenotypic plasticity. By contrast, genotypes adapted to unfavorable conditions, may have superior performance in restrictive environments but are unable to take advantage of favorable conditions, leading to a low plasticity. In this study, the families developed in the Eight Region Sandy Soils sites exhibited behavior consistent with this theory, *i.e.* have a higher growth in water restriction, but less growth in favorable conditions. These families, which grew always under restricted climate environments (*i.e.* low water holding capacity and high vapor pressure deficit) (Table 1), are not able to take advantage of favorable environmental conditions represented by the well-watered treatment. This could indicate a more conservative strategy of water use in these families when faced drought, allocating more roots at the expense of shoots, strategy that could have been influenced by environmental pressures in which these families developed. On the other hand, families of the coastal sites (*e.g.* Eight Region Coastal sites) have the highest values of plasticity and biomass allocated to shoots.

In general, the results obtained in this study agree with those from other species (*e.g.* Chambel *et al*., 2007), which confirms the reliability of the results obtained in short-term tests with artificially imposed stresses. However, in the case of families belonging to Eight Region Fothills of the Andes sites, results must be interpreted with caution because only two of the five families initially sowed (Table 1) survived with more than 15 seedlings each in water stress treatment, and high standard deviation (Table 4) could have biased re-

sults. Only 60 seedlings were analyzed in these families, while in the case of families from Eight Region Coastal sites, 570 seedlings were analyzed. Although the water stress treatment applied maybe too short to obtain informative data on drought regulation mechanisms at organ and whole plant level, the drought period was long enough to affect morphological mechanisms regulating drought responses. For example, survival was negatively affected, reaching only 44% in the waters-stress treatment ($p < 0.01$), with families from Seventh Region Coastal and Eight Region Sandy Soils sites reaching the highest percentages, which is coincident with the separation of families in different groups, those belonging to coastal sites and those belonging to interior sites (specifically Sandy Soil sites), denoting a significant pattern of genetic variation that can also be considered as plasticity for survival.

Resource allocation patterns may change with plant size, *i.e.* they may be "allometric" in the broad sense, and it has been argued that some observed changes in allocation are primarily due to size (Coleman *et al*., 1994). Part of the ontogenetic drift is related to the size of the plant, as larger plants will have to invest a larger fraction of their biomass in support structure, and have a larger leaf area so that self-shading increases. In this study seedlings from all families were in the same stage of development, *i.e.* before the formation of dwarf shoots, which support the fact that differences between families were attributable to phenotypic plasticity (Poorter and Nagel, 2000) rather than ontogenetic plasticity (Wright and McConnaughay, 2002). Methods used in this study to analyze plant biomass allocation assume that the outcome of interactions is size independent. The technique of the allometric analysis allowed us to correct allocation patterns for possible size differences between plants of different treatments. When height of plants was analyzed as a covariate, results support this, *i.e.* plants size did not influenced neither growth nor biomass allocation.

Although it is debatable whether the emphasis in tree breeding should be on producing genotypes suitable for specific environments, or genotypes suited to a wide range of environments, family X watering interaction found in this study could help to select families according to their drought tolerance. Since drought events are predicted to increase in the long term for Central Chile (CONAMA, 2009), negatively affecting establishment and productivity of radiata pine, families from Sandy Soils Eight Region could be established in more drought prone areas, because as was seen, they had high investment to roots and low investment to needles (transpiring organs) which conforms to a drought tolerant phenotype as it would allow both maximum water capture and minimum water losses. Also, and as was reported by Espinoza, (2012) for the same families unpublished data, family X watering interaction was not too strong to reduce heritability and thus, effectiveness of selection. Survival and probably productivity of radiata pine in drought prone sites could thus be increased by breeding for high root biomass and low shoot biomass.

Finally, the results of this study allows us to speculate that the novel environmental conditions that were experienced by radiata pine upon its introduction to a new habitat in Chile, might have favored more phenotypically plastic responses and result in an increased ability to success in new environment. It is likely that the intensity of natural selection experienced in the first generations after planting in 1891, has been low, since it was introduced in a coastal area with soil and climatic characteristics similar to the natural range of the species (*i.e.* Eighth Region Coastal sites). Colonizers are frequently thought to have high phenotypic plasticity, permitting phenotypic variation of a particular genotype under different environmental challenges, and providing tolerance over a range of environmental conditions (Bradshaw, 1965; Thompson, 1991). Once the species was established in new environments (*i.e.* Eighth Region Sandy Soils sites), new plantations have experienced different selection pressures, resulting in adaptation to new conditions. It has been hypothesized that differing selection pressure on novel phenotypes could lead to local adaptation (Pigliucci *et al*., 2006) and fast formation of local land races. Seedlings from seeds harvested in planted stands with transferred provenances perform differently from seedlings of their original provenance. In Denmark, seedlings from seeds harvested in first generation stands of *Picea sitchensis* have better survival and are less injured by climate than seedlings of the same provenances from direct import from North America (Nielsen, 1994). Similar experiences are found with *Abies normaniana* in Denmark (Nielsen, 1999). In Scotland, seedlings from first generation *Abies grandis* stands perform far better than direct import of the same provenance (Ennos *et al*., 1998). In Germany, Douglas fir seedlings from German stands perform better than foreign provenances (Kleinschmit *et al*., 1974). Thus, a fast formation of "land races" (from one generation to the next) seems to occur in several conifer species.

Acknowledgments

We gratefully acknowledge Dr Verónica Emhart from Forestal Mininco S.A. and Rodolfo Calquín from Forestal Celco S.A. for supplying the seeds to carry out this experiment. Financial support was granted by the Dryland Technological Center (Innova Chile Contract N° 06FC01 IXC-96). Many thanks also to Dr Rómulo Santelices, Director of the Dryland Technological Center for supporting this research. Thanks to Jaime Araya, Italo Pérez and Cristian Pacheco for their support in the nursery measurements.

References

Albert F, 1900. Las dunas o sean las arenas volantes, voladeros, arenas muertas, invasión de las arenas, playas i médanos del centro de Chile. Comprendiendo el litoral desde el norte de la provincia del Aconcagua hasta el límite sur de la de Arauco. Cervantes. Santiago, Chile.

Atzmon N, Moshe Y, Schiller G, 2004. Ecophysiological response to severe drought in *Pinus halepensis* Mill. trees of two provenances. Plant Ecology 171: 15-22.

Bahamondez C, Martín M, Müller-Using S, Pugin A, Vergara G, Rojas Y, 2010. Estudio de la variabilidad de los bosques de Chile al cambio climático. V Congreso Chileno de Ciencias Forestales. Diálogos entre la Ciencia y la Sociedad. Temuco, Chile, Octubre 2010. p: 47.

Behera N, Nanjundiah V, 2004. Phenotypic plasticity can potentiate rapid evolutionary change. Journal of Theoretical Biology 226: 177-184.

Balaguer L, Martínez-Ferri E, Valladares F, Pérez-Corona ME, Baquedano FJ, Castillo FJ, Manrique E, 2001. Population divergence in the plasticity of the response of *Quercus coccifera* to the light envionment. Functional Ecology 15: 124-135.

Bloom AJ, Chapin FS, Mooney HA, 1985. Resource limitation in plants – an economic analogy. Annual Review of Eco-logy and Systematics 16: 363-392.

Bradshaw AD, 1965. Evolutionary significance of phenotypic plasticity in plants. Advances in Genetics 13: 115-155.

Calamassi R, Della Rocca G, Falusi M, Paoletti E, Strati S, 2001. Resistance to water stress in seedlings of eight European provenances of *Pinus halepensis* Mill. Annals of Forestry Science 58: 663-672.

Camus P, 2006. Ambiente, bosques y gestión forestal en Chile 1541-2005. Diban LOM. Santiago, Chile.

Coleman JS, McConnaughay KDM, Ackerly DD, 1994. Interpreting phenotypic variation in plants. Trends in Ecology and Evolution 9: 187-191.

CONAMA, 2009. Análisis de vulnerabilidad del sector silvoagropecuario, recursos hídricos y edáficos de Chile frente a escenarios de cambio climático. Capítulo: "Impactos productivos en el sector silvoagropecuario de Chile frente a escenarios de cambio climático". Gobierno de Chile, CONAMA-ODEPA-FIA. Ejecutor: Centro de Agricultura y Medio Ambiente (AGRIMED) Facultad de Ciencias Agronómicas. Universidad de Chile.

Corcuera L, Gil-Pelegrin E, Notivol E, 2010. Phenotypic plasticity in *Pinus pinaster* $\delta^{13}C$: environment modulates genetic variation. Annals of Forest Science 67: 812.

Chambel MR, Climent J, Alía R, 2007. Divergence among species and populations of Mediterranean pines in biomass allocation of seedlings grown under two watering regimes. Annals of Forest Science 64: 87-97.

Danusevičius D, Persson B, 1998. Phenology of natural swedish populations of *Picea abies* as compared with introduced seed sources. Forest Genetics 5(4): 211-220.

Dean CA, Sands R, 1983. Stomatal response to evaporative demand and soil water status in families of Radiata pine. Australian Forest Research 13: 179-182.

Ennos R, Worrell R, Malcolm DC, 1998. The genetic management of native species in Scotland. Forestry 71: 1-23.

Espinoza S, 2012. Caracterización de la raza local de *Pinus radiata* D. Don en Chile en relación a su diversidad genética y respuesta temprana frente a una restricción hídrica. Tesis doctoral. Universidad de Chile. 115 pp.

Gerding V, Schlatter J, 1995. Variables y factores del sitio de importancia para la productividad de *Pinus radiata* D. Don en Chile. Bosque 16(2): 39-56.

Huber A, Trecaman R, 2002. Efecto de la variabilidad interanual de las precipitaciones sobre el desarrollo de plantaciones de *Pinus radiata* (D. Don) en la zona de los arenales VIII Región, Chile. Bosque 23(2): 43-49.

Hood JV, Libby WJ, 1980. A clonal study of intraspecific variability in Radiata pine I. Cold and animal damage. Australian Forest Research 10: 9-20.

INFOR, 2010. Anuario Forestal 2010. Instituto Forestal, Chile. Boletín Estadístico N° 128. 134 pp.

Kaufman SR, Smouse PE, 2001. Comparing indigenous and introduced populations of *Melaleuca quinquenervia* (Cav.) Blake: response of seedlings to water and pH levels. Oecologia 127: 487-494.

Kleinschmit J, Racz J, Weissgerber H, Dietze W, Dieterich H, Dimpflmeier R, 1974. Ergeb-nisse aus dem internationalen Douglasien-herkunftversuch von 1970 in der Bundesrepublik Deutschland. Silvae Genetica 23: 167-176.

Klinkhamer PGL, Jong TJD, Meelis E, 1990. How to test for proportionality in the reproductive effort of plants. American Naturalist 135: 291-300.

Lei Y, Yin CH, Li CH, 2006. Differences in some morphological, physiological, and biochemical responses to drought stress in two contrasting populations of *Populus przewalskii*. Physiologia Plantarum 127: 182-191.

Lewis NB, Ferguson IS, 1993. Management of Radiata pine. In: The Chilean radiata pine sector (Sutton WR, Donald MG, Lisboa HB, eds). Inkata Press. Melbourne, Australia. pp: 365-379.

Lortie CJ, Aarssen LW, 1996. The specialization hypothesis for phenotypic plasticity in plants. International Journal of Plant Sciences 157: 484-487.

Merchant A, Callister A, Arndt S, Tausz M, Adams M, 2007. Contrasting physiological responses of six *Eucalyptus* species to water deficit. Annals of Botany 100: 1507-1515.

Nicotra AB, Atkin OK, Bonser SP, Davidson AM, Finnegan EJ, Mathesius U, Poot P, Purugganan MD, Richards CL, Valladares F, Van Kleunen M, 2010. Plant phenotypic plasticity in a changing climate. Trends in Plant Science 15: 684-692.

Nielsen UB, 1994. Genetic variation in Sitka Spruce (*Picea sitchensis* (Bong.) Carr.) with respect to height growth, stem form and frost hardiness – investigated on the basis of Danish provenance, progeny and clonal field experiments. Forskingsserien. Danish Landscape and Forest Research Institute, Hørsholm, Denmark 11: 1-330.

Nielsen UB, 1999. Comparison of Danish first generation or later seed sources with direct imports – examples from Sitka spruce, Nordmanns fir and Noble fir. Aktuelt Fra Skogforskningen 3: 11.

Pigliucci M, Schlichting CD, 1996. Reaction norms of *Arabidopsis*. IV. Relationships between plasticity and fitness. Heredity 76: 427-436.

Pigliucci M, Murren CJ, Schlichting CD, 2006. Phenotypic plasticity and evolution by genetic assimilation. The Journal of Experimental Biology 209: 2362-2367.

Poorter H, Nagel O, 2000. The role of biomass allocation in the growth response of plants to different levels of light, CO_2, nutrients and water: a quantitative review. Australian Journal of Plant Physiology 27: 595-607.

Rowell DM, Ades PK, Tausz M, Arndt SK, Adams MA, 2009. Lack of genetic variation in tree ring $\delta^{13}C$ suggests a uniform, stomatally-driven response to drought stress across *Pinus radiata* genotypes. Tree Physiology 29: 191-198.

Samson DA, Werk KS, 1986. Size-dependent effects in the analysis of reproductive effort in plants. American Naturalist 127: 667-680.

SAS Institute. 1999. SAS/STAT user's guide, Version 8. SAS Institute INC. Cary, NC. 3848 pp.

Scheiner SM, 1993. Genetics and evolution of phenotypic plasticity. Annual Review of Ecology and Systematics 24: 35-68.

Schlichting CD, Pigliucci M, 1998. Phenotypic evolution - A reaction norm perspective. Sinauer Associates. Sunderland, MA.

Simpson JA, Ades PK, 1990. Screening *Pinus radiata* families and clones for disease and pest insect resistance. Australian Forestry 53: 194-199.

Squire R, Neales T, Loveys B, Attiwill P, 1988. The influence of water deficits on needle conductance, assimilation rate and abscisic acid concentration of seedlings of *Pinus radiata* D. Don. Plant Cell and Environment 11: 13-19.

Thompson JD, 1991. Phenotypic plasticity as a component of evolutionary change. Trends in Ecolgy and Evolution 6: 246-249.

Toro J, Gessel S, 1999. Radiata pine plantations in Chile. New Forests 18(1): 33-44.

Via S, 1993. Adaptive phenotypic plasticity – Target or by-product of selection in a variable environment. American Naturalist 142: 352-365.

Wright S, Mcconnaughay K, 2002. Interpreting phenotypic plasticity: the importance of ontogeny. Plant Species Biology 17: 119-131.

Zhang X, Zang R, Li CH, 2004. Population differences in physiological and morphological adaptations of *Populus davidiana* seedlings in response to progressive drought stress. Plant Science 166: 791-797.